Medical Microbiology and Immunology (2019) 208:39–48
https://doi.org/10.1007/s00430-018-0556-z

ORIGINAL INVESTIGATION

β-Glucan induces autophagy in dendritic cells and influences T-cell differentiation

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Received: 6 June 2018 / Accepted: 4 August 2018 / Published online: 7 August 2018

Abstract

β-Glucan has been reported to activate dendritic cells (DCs), and activated DCs, subsequently, promote Th1 and cytotoxic T-lymphocyte priming and differentiation in vitro. However, the mechanism that regulates the immune response of β-glucan-induced DCs has not been thoroughly elucidated to date. Recent studies have drawn attention to a strong relationship between pathogen-associated molecular patterns (PAMP) recognition and autophagy for the activation of DC function. In this study, we observed that β-glucan induced the expression of a number of autophagy-related genes and the formation of autophagosomes in DCs. To further investigate whether β-glucan-induced DC activation and innate cytokine production are associated with autophagy, we utilized 3-MA to block autophagosome formation and accessed the maturation and function of DCs induced by β-glucan. We found that autophagy-deficient DCs showed downregulated expression of MHC-II and CD80, decreased TNF-α secretion, and reduced production of iNOS upon β-glucan stimulation. Further examination demonstrated that blockade of autophagy in β-glucan-induced DCs significantly attenuated IFN-γ production by co-cultured CD4 + T cells and inhibited the proliferation and differentiation of CD4 + T cells. Thus, these data indicate that autophagy in β-glucan-induced DCs is a crucial mechanism for the maturation of DCs, and it drives innate cytokine production, thereby facilitating adaptive immune responses.

Keywords β-Glucan · Dendritic cells · Autophagy · T-cell differentiation

Introduction

β-Glucan, as a biological response modifier, was first described 45 years ago, and has been extensively investigated for both its anti-tumor and anti-infective activities [1–3]. β-Glucan has been recognized as a major fungal pathogen-associated molecular pattern (PAMP), which can strongly influence natural and adaptive host immune responses, mostly through engagement of the C-type lectin receptor, dectin-1 [4]. Most β-glucans are derived from yeast, bacteria, barley, or fungi, and have a backbone structure of linear β-1,3-linked D-glucose molecules (β-1,3-D-glucan). β-Glucans also have β-1,6-linked side chains of β-1,3-D-glucan of varying sizes that occur at different intervals along the backbone [5]. Recent studies have also demonstrated that β-glucan can function as a potent adjuvant to stimulate innate and adaptive immune responses [6, 7]. Dectin-1, as a non-Toll-like pattern recognition receptor for β-glucan, was originally reported as a DC-specific molecule with a T-cell co-stimulatory capacity, but later, its expression was found to be stronger in monocytes, macrophages, and neutrophils. When expressed on polymorphonuclear leukocytes, mononuclear cells, macrophages, and DCs, the dectin-1 receptor mediates the biological effects of β-glucan, including glucan-dependent anti-cancer immune responses. In murine tumor models, we found that orally administered particulate yeast-derived -glucans (WGP) elicits potent anti-cancer

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00430-018-0556-z) contains supplementary material, which is available to authorized users.

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immune responses, leading to delayed tumor progression. Stimulation with WGP led to DC maturation and cytokine secretion, promoted CD4+ T cells to Th1 differentiation, and induced tumor-specific CTLs [8, 9]. In addition, we also found that WGP had similar effects on human monocyte-derived DCs and induced CD8+ IFN-γ + T-cell immune responses through the PI3K/AKT pathway [10]. However, the exact mechanisms of the anti-cancer immune response induced by WGP-induced DCs have not been thoroughly elucidated to date.

The autophagy system is a critical cellular response that facilitates self-digestion of misfolded or unused protein and cellular debris and impacts development, aging, and normal function of cellular processes. Induction of autophagy can directly impact the immune system by optimizing the activation of antigen-presenting cells (APC) and other innate immune cells for immediate cytokine responses and activation of T cells through increased antigen presentation [11–13]. In fact, autophagy has been identified not only in the delivery of antigens for MHC class II presentation to CD4+ T cells but also for cross-presentation of antigens for CD8+ T-cell activation. In addition, immune responses differentially regulate autophagy, with Th1 promoting and Th2 inhibiting the process for the activation of innate immune cells [14]. Recent findings have suggested clear links between autophagy and Toll-like receptors (TLR), as well as other pathogen receptor recognition (PRR) activation, such as NOD2 [15, 16]. The previous studies have established that PRR/TLR-mediated activation in DCs can induce autophagy and enhance the immune response. A family of autophagy-related genes orchestrates the initiation, elongation/closure, and maturation of autophagosomes, including ATG5, ATG7, ATG8/LC3, and the conjugation of free cytosolic LC3 (LC3-I) to phosphatidylethanolamine (LC3-II), which is an important process of autophagy. It has been reported that LC3 is, indeed, recruited to phagosomes containing exogenous zymosan particles in DCs [17].

Whether β-glucan can induce DCs or the effect of autophagy in β-glucan-induced DCs' immunity remains to be determined. In this study, we demonstrate that autophagy is involved in β-glucan-induced DC maturation and induction of the CD4+ IFN-γ+ T-cell immune response. In addition, the inhibition of autophagy by 3-MA alters the immune response of β-glucan-induced DCs through the regulation of IFN-γ production by CD4+ T cells. Taken together, these findings expand our understanding of the innate immune response of DCs induced by β-glucan and supply a new area of study for DC-based vaccine optimization for cancer therapeutics.

Materials and methods

Preparation of β-glucans

WGP β-glucan (generously provided by Prof. Jun Yan, University of Louisville) was purified from the cell walls of *Saccharomyces cerevisiae*. A series of alkaline and acid extractions yielded hollow yeast cell wall "ghosts" composed primarily of long β-1,3 glucose polymers, with 3–6% of the backbone glucose units possessing a β(1,6) branch (β-(1,3/1,6)-D-glucan). WGPs were hydrated in distilled water and sonicated to produce a single-particle suspension. To remove any trace amounts of LPS contamination, the WGPs were suspended in 200 mM NaOH for 20 min at room temperature (RT), washed thoroughly, and resuspended in LPS-free water as described previously. The endotoxin level was 0.06 EU/mL as tested by the gel-clot method (Associates of Cape Cod, East Falmouth, MA).

Mice

C57BL/6 mice, 6–8 weeks old, were purchased from Changzhou Cavens Lab Animals. CD4+ ovalbumin T-cell receptor transgenic (Tg) OT-II mice were generously provided by Prof. Hai Qi, Tsinghua University. All mice were maintained under specific pathogen-free conditions and used at 6–8 weeks of age.

Reagents

Recombinant mouse GM-CSF, IL-4, apoptosis detection kit, and ELISA kits for murine IL-12p70, IL-10, IL-6, TNF-a, and IFN-γ were purchased from Biolegend. Fluorescein-conjugated mAbs against CD11c (Clone N418), MHC-II (Clone M5/114.15.2), CD40 (Clone HM40-3), CD80 (Clone 16-10A1), CD86 (Clone GL-1), CD4 (Clone GK1.5), and FOXP3 (Clone MF-14), isotype control mAbs, carboxyfluorescein diacetate succinimidyl ester (CFSE), and GolgiPlug were purchased from BD Pharmingen. Microbead-conjugated mAbs against CD4 and CD11c were purchased from Miltenyi Biotec. 3-Methyladenine (3-MA) purchased from Sigma-Aldrich was reconstituted with PBS + 0.1% BSA and used at 3 mM in cell treatments. Anti-LC3B (Clone D11, Cat. no. 3868), anti-p62 (Cat. no. 5114), and anti-β-actin (Clone 13E5, Cat. no. 4970) were obtained from Cell Signaling Technology; anti-beclin-1 (Cat. no. PA1-16857) was obtained from Thermo Fisher Scientific and anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (Cat. no. ab97051) were obtained from

Abcam. Ovalbumin peptide (323–339) (Cat. no. O1641) was purchased from Sigma-Aldrich.

Bone marrow dendritic cell cultures

BMDCs were cultured from whole bone marrow obtained from C57BL/6 mice as indicated. Bone marrow cells harvested from C57BL/6 mice were cultured in complete RPMI 1640 medium (Gibco Life Technologies, Breda, The Netherlands) containing L-glutamine, sodium pyruvate, nonessential amino acids, and 2-mercaptoethanol supplemented with 10% FCS, 20 ng/mL GM-CSF, and 5 ng/mL IL-4. On day 5, the dendritic proliferating clusters were collected and purified by anti-CD11c microbeads as immature BMDCs (imDCs). The purity of DCs was > 93%, as confirmed by FACS. The imDCs were cultured for an additional 2 days with particulate β-glucan WGPs (100 μg/mL) or without stimuli as a control. Simultaneously, imDCs were also pretreated with 3 mM of 3-MA 1 h prior to WGP stimulation for 2 days. The resulting DCs were thoroughly washed and used for phenotypical (flow cytometry) and functional characterization (co-culture assays), and supernatants were frozen for cytokine evaluation by ELISA.

Immunofluorescence staining

Isolated imDCs were stimulated by WGP for 24 h with or without 3-MA. Later, the cells were harvested and surface stained with anti-CD11c mAbs for 30 min, fixed in 4% paraformaldehyde for 20 min, permeabilized with 01% Triton-X-100 for 5 min, and blocked in 5% BSA with 01% Tween-20. The cells were incubated for 2 h with primary antibody against LC3B (D11). Subsequently, the cells were incubated with a corresponding fluorescence-labeled secondary antibody for 1 h and were viewed on a microscope (Olympus IX71, Center Valley, PA).

Western blot analysis

Western blotting was performed as described previously [19]. A total of 1×10^7 cells were washed with PBS and resuspended in 1 mL ice-cold RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with freshly added protease inhibitors (10 mg/mL PMSF, 30 mg/mL aprotinin, and 100 mM Na_3VO_4). The preparation was transferred to microcentrifuge tubes and centrifuged at 10,000*g* for 10 min at 4 C. The supernatant was subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes according to the manufacturer's protocols (Bio-Rad Corp, USA). After blocking, the membrane was incubated with primary antibodies against LC3B, beclin-1, P62, and β-actin. Proteins were detected with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG and an enhanced chemiluminescence detection system (GE Life Sciences, Pittsburgh, PA).

Phenotype analysis of BMDCs by flow cytometry

Cells (4×10^5) in 100 mL PBS were blocked with Fc-blocking monoclonal antibody for 15 min on ice. Cells were stained with fluorochrome-tagged monoclonal antibodies against CD11c, CD40, CD80, CD86, MHC-II, or isotype controls (eBioscience, San Diego, CA) on ice for 30 min, washed with ice-cold staining buffer, fixed with 2% paraformaldehyde in PBS, and analyzed by flow cytometry (FACSCanton II, BD Biosciences, San Jose, CA).

Enzyme-linked immunosorbent assay

After treatment, the cell culture medium was collected and centrifuged at 10,000 rpm for 5 min. IL-2, IL-6, TNF-a, IL-10, and IL-12p40 concentrations were determined by enzyme-linked immunosorbent assays (ELISA, Biolegend) according to the manufacturer's instructions.

qRT-PCR

Cells were treated with TRIzol reagent (Invitrogen), and total RNA was isolated and reverse transcribed with TaqMan Reverse Transcription Reagents (Applied Biosystems). The indicated cytokine mRNA levels were quantified by quantitative RT-PCR (qRT-PCR) amplification using the Bio-Rad MyiQ single color RT-PCR detection system. Briefly, complementary DNA was amplified in a 25 μL reaction mixture containing 12.5 μL of SYBR Green PCR supermix (Invitrogen), 100 ng of complementary DNA template, and selected primers (200 nM) using the recommended cycling conditions. Data were acquired on an ABI ViiA 7 real-time PCR system. The primer sequences, designed with Primer Express Software Version 2.0 (Applied Biosystems), are summarized in Supporting Information Table 1.

T-cell differentiation, priming, and proliferation assay

CD4+ T cells from the lymph nodes and spleens of OT-II mice were purified with magnetic-activated cell-sorting beads. Immature BMDCs were incubated with WGP for 48 h, either with or without 3-MA, washed to clear residual 3-MA, and, subsequently, co-cultured for 3 days with Purified OT-II T cells at a 1:10 ratio in the presence of 50 μg/mL of ovalbumin (OVA) protein in complete RPMI 1640 culture medium containing 10% FBS supplemented with sodium pyruvate, L-glutamine, nonessential amino acids, and 2-mercaptoethanol. On day 3, supernatants from the cell culture medium were collected to detect the secretion of IFN-γ by

ELISA. For the Foxp3 differentiation assay, the cells were restimulated with PMA and ionomycin in the presence of GolgiPlug, and the cells were subsequently surface stained with anti-CD4 mAbs, fixed, permeabilized, and stained with anti-Foxp3. For the proliferation assay, the T cells were pre-labeled with CFSE and cultured with the DCs. Data were acquired by flow cytometry on a FACS Canton II (BD Biosciences, San Jose, CA) and analyzed by FlowJo software Version 8.7 (TreeStar, Ashland, OR).

Statistics

Statistical significance between groups was determined by two-tailed Student's *t* test. The data are presented as the means ± SD. Significance was accepted at $p < 0.05$.

Results

WGP induces autophagy in BMDCs

The previous reports have shown that β-glucan can activate robust autophagy-dependent protein expression in macrophages. To investigate a link between β-glucan (WGP) and autophagy induction, we used bone marrow-derived immature CD11c + DCs induced by WGP for 24 h, and we measured the expression of the classic autophagy-associated genes ATG5, ATG7, LC3B, and beclin-1. After WGP treatment of BMDCs, we observed that the expression of LC3B and beclin-1 was highly upregulated, although the expression of ATG5 or ATG7 was not clearly changed in comparison with non-stimulated cells (Fig. 1a). The conversion of LC3-I to LC3-II is a well-known hallmark of autophagy induction, and LC3-II is localized in the inner and outer membranes of autophagosomes, which is degraded after the fusion of autophagosomes with lysosomes. We found that WGP increased LC3B-II expression of BMDCs compared to

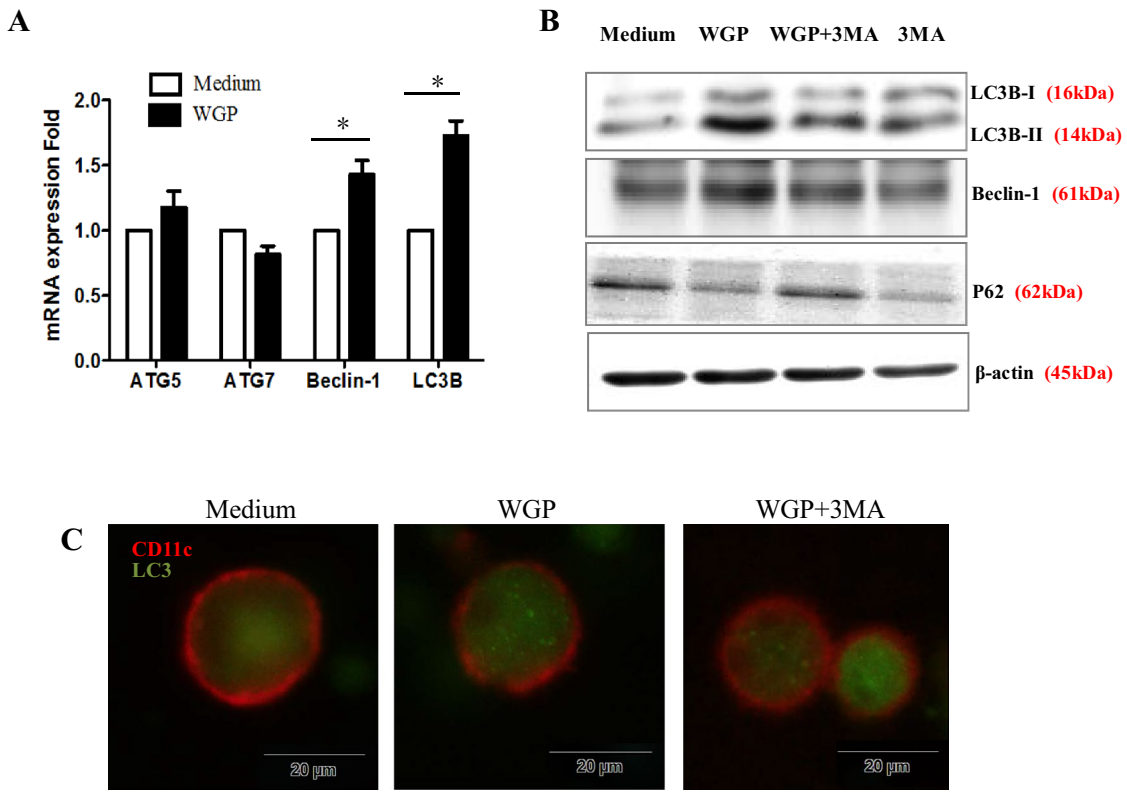

Fig. 1 WGP stimulation induces autophagy in bone marrow-derived dendritic cells (BMDCs). **a** Mouse immature BMDCs were generated by culturing in 20 ng/mL GM-CSF and 5 ng/mL IL-4 for 5 days. The imDCs were then purified and stimulated with WGP (100 μg/mL). **a** After 24 h, autophagy protein genes were assessed by qRT-PCR. The results are presented as mean ± SD of triplicates from one of three experiments with similar results. **b** imDCs were treated with 3MA(3 mM) 30 min prior to the addition of WGP; the level of LC3-I, LC3-II, beclin-1, and p62 proteins was detected by Western blot, and **c** LC3B-positive puncta in BMDCs were examined for identifying autophagosome formation by fluorescence staining. Cells were stained with antibody to LC3 and antibody to CD11c+. The asterisk (*) indicates $p < 0.05$

non-stimulated cells and that the level of beclin-1 protein in WGP-induced BMDCs was also upregulated, as detected by Western blot analysis. Along with the higher expression of LC3B-II, the level of p62 protein was decreased, suggesting that autophagic flux in WGP-induced BMDCs was unhindered (Fig. 1b). Next, we confirmed our results by examining the degree of autophagosome formation induced by WGP treatment of BMDCs by immunofluorescent staining, and we observed increased autophagosome formation as green puncta in WGP-induced BMDCs (Fig. 1c). However, the induction of autophagy mediated by WGP could be inhibited by addition of the autophagy inhibitor 3-MA (3 mM) to the cultures (Fig. 1b, c). These results suggest that WGP is capable of directly inducing autophagosome formation in BMDCs.

Effect of autophagy on the phenotypic maturation of WGP-induced BMDCs

Because autophagy was induced in BMDCs after WGP treatment, we wanted to determine whether autophagy affects the WGP-induced phenotypic maturation of BMDCs. We pretreated BMDCs with or without 3-MA 1 h prior to addition of WGP and evaluated the maturation markers CD11c, MHC-II, CD86, CD80, and CD40 using flow cytometry. The cell viability is not significantly altered on BMDC with or without 3MA only stimulation (Supplemental Fig S1). The WGP-induced, 3-MA-pretreated BMDCs expressed significantly lower levels of MHC-II and CD86 compared to WGP-induced BMDCs without 3-MA, and the expression of CD80 and CD40 was slightly downregulated in WGP-induced BMDCs with 3-MA, though this difference was not statistically significant (Fig. 2). These results suggest that autophagy plays a critical role in the maturation of BMDCs stimulated by WGP.

Effect of autophagy on pro-inflammatory cytokines produced by WGP-induced BMDCs

To determine the effect of autophagy on the pro-inflammatory cytokines produced by WGP-induced BMDCs, we assessed the presence of inflammation-related cytokines by ELISA and qRT-PCR. TNF-α is an important cytokine secreted by mature and activated DCs to initiate the Th1 immune response. iNOS can affect the signaling pathway of mature DCs. As shown in Fig. 3, the secretion of TNF-α in WGP-induced BMDCs was significantly reduced in the presence of 3-MA, as measured by mRNA and protein levels. In addition, 3-MA treatment clearly decreased the expression of iNOS mRNA (Fig. 3a), but did not affect the secretion of IL-12 in WGP-induced BMDCs (Fig. 3b). Overall, these data suggest that autophagy inhibition hindered some pro-inflammatory cytokine secretion by WGP-induced DCs.

Autophagy is involved in Th1 priming and Treg differentiation induced by WGP-DCs

As mentioned above, autophagy can regulate the maturation of BMDCs induced by WGP. Therefore, we investigated whether WGP-induced autophagy in BMDCs exhibits any effect on priming CD4 + T cells. BMDCs were incubated with WGP in the presence or absence of 3-MA for 48 h, washed to clear residual 3-MA, and subsequently co-cultured with ovalbumin-specific naive CD4 + T cells from OT-II mice. After 3 days, CD4 + T-cell proliferation was measured by a CFSE dilution assay, and the supernatants were harvested to assay IFN-γ production. As shown in Fig. 4, we found that the addition of WGP drastically increased CD4 + T-cell proliferation and increased the IFN-γ produced by CD4 T cells. Treg differentiation was significantly decreased by WGP stimulation. The adjuvant effect of WGP was mediated by DCs but not T cells, as the T cells did not proliferate when they were stimulated with WGP alone (data not shown). However, when DCs were incubated with the autophagy inhibitor 3-MA prior to WGP treatment, we observed that the DCs showed a significant decline in the proliferation of CD4 + T cells and the secretion of IFN-γ by CD4 + T cells (Fig. 4a, b). Decreased ability to induce T-cell proliferation and differentiation could be due to the decreased expression of MHC-II and co-stimulatory molecules, rather than impaired antigen presentation. Then, OT-II T cells were induced by the DCs pulsed with MHC-II restricted T-cell epitope of ovalbumin. We found that 3-MA also inhibited CD4 + T proliferation and IFN-γ secretion by the DCs pulsed with ovalbumin peptide (Supplemental Fig S2a, S2b). Meanwhile, we found that 3-MA could reverse the proportion of Treg cells stimulated by WGP-activated BMDCs and increase Treg differentiation (Fig. 4c). In summary, autophagy induced by WGP promoted the capacity of DCs to increase naive CD4 + T-cell differentiation into Th1 and decreased Treg differentiation.

Discussion

DCs are professional antigen-presenting cells and can trigger the T-cell activation required for the initiation and modulation of immune responses. Importantly, the efficacy of DCs depends on many variables, especially maturation status and efficient antigen presentation to naive T cells. Recent studies have demonstrated that β-glucans can function as a potent adjuvant to stimulate innate and adaptive immune responses. Previously, we found that yeast-derived whole β-glucan particles (WGPs) can activate and mature murine/human DCs both in vitro and in vivo, leading to enhanced Ag-specific CD4 + and CD8 + T-cell responses. However, we still do not understand the mechanisms underpinning the impact

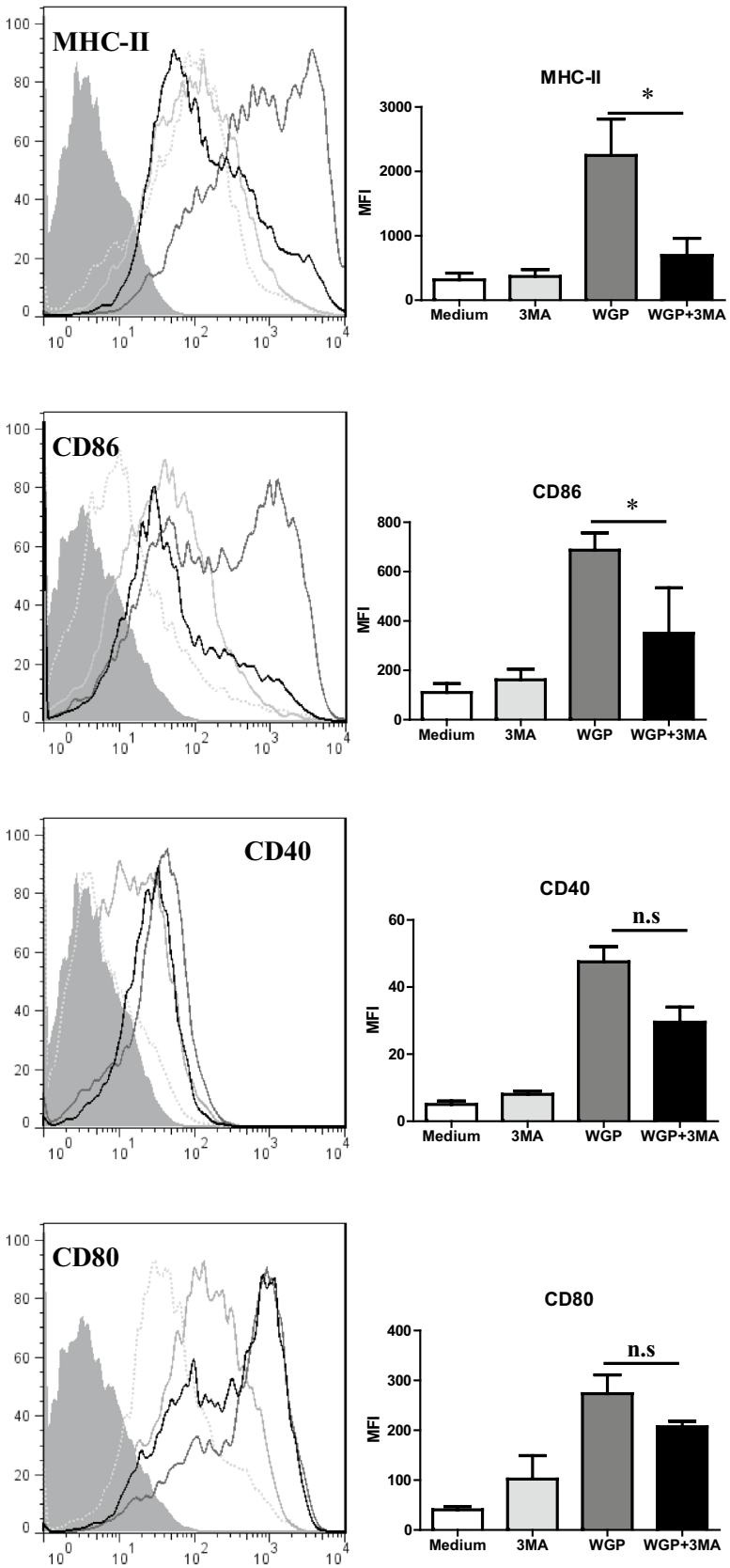

Fig. 2 Autophagy affected the phenotype of BMDCs matured by WGP. The imDCs were stimulated with WGP for 48 h, either with or without autophagy inhibitor 3MA. Surface marker expression was assessed by flow cytometry. The shaded peaks represent isotype controls; the dashed line represents medium only; the light grey line represents 3MA addition; the grey line represents WGP stimulation; the dark line represents WGP stimulation with 3MA. Data shown are the mean of triplicates ± SD from one representative experiment and reproducible in three independent experiments. The asterisk (*) indicates $p < 0.05$ and n.s indicates no significant difference. $p < 0.05$ or n.s for comparisons between WGP with or without 3MA for both graphs

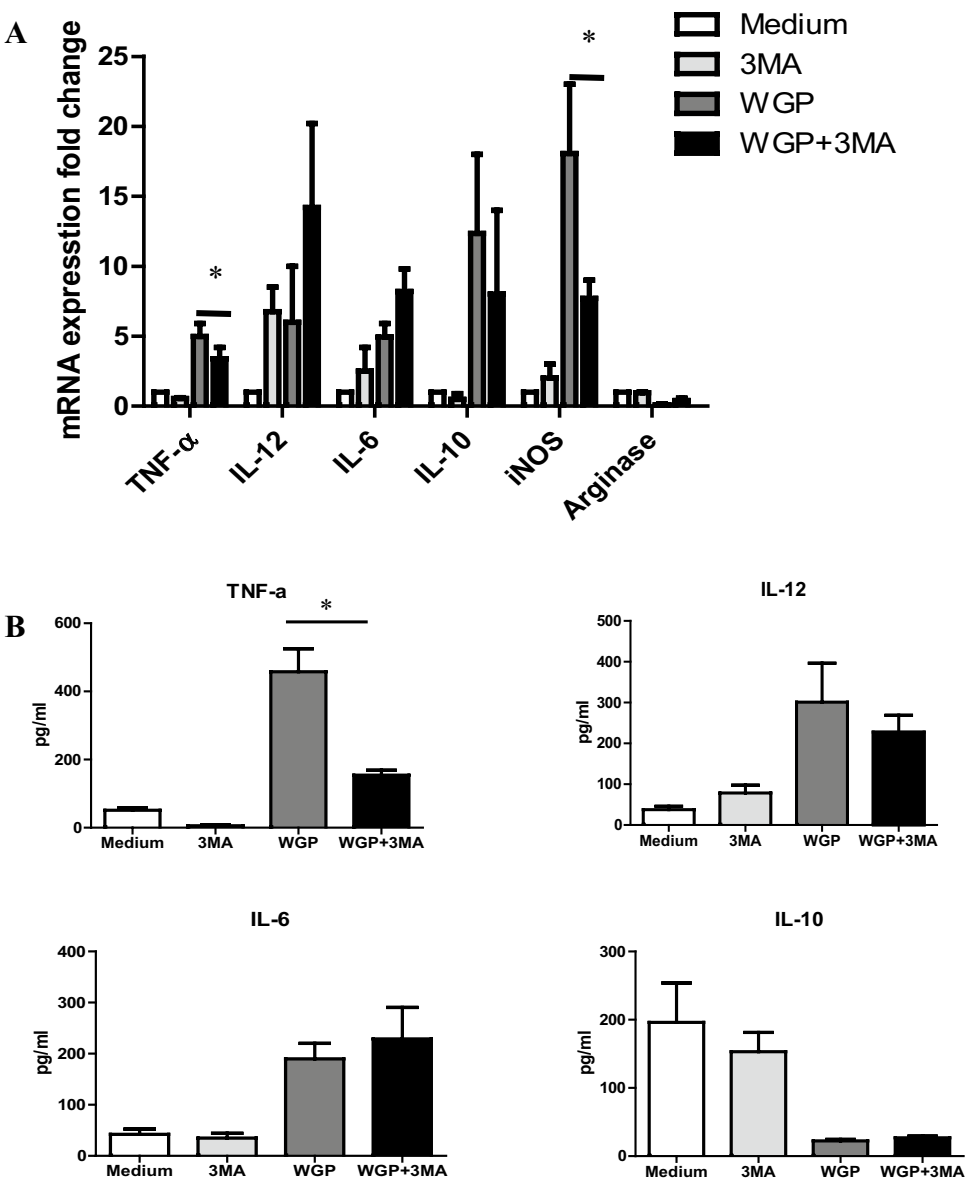

Fig. 3 Autophagy affected the cytokine production by BMDCs stimulated with WGP. Immature BMDCs were stimulated with WGP for 48 h, either with or without autophagy inhibitor 3MA. a Supernatants from cell culture medium were collected and assayed by ELISA. b RNAs from BMDCs induced by different stimuli were extracted, and qRT-PCR was performed for the indicated cytokines. The results are presented as mean ± SD from three independent experiments. The asterisk (*) indicates $p < 0.05$ for comparisons between WGP with or without 3MA for both graphs

of WGP on the immune function of DCs. In this study, we found that autophagy was involved in the maturation and T-cell priming stimulated by WGP-induced BMDCs.

There are three different forms of autophagy in eukaryotic cells, including chaperone-mediated autophagy, macroautophagy, and microautophagy [18–20]. Macroautophagy has been implicated in innate and adaptive immunity. This process contributes to innate immune responses by participating in the capture, degradation, and eradication of intracellular pathogens, such as bacteria, protozoa, and viruses [21–23], and to adaptive immune responses through endogenous and exogenous antigen processing for MHC class II presentation and probably MHC class I presentation [24–27]. Accumulating evidence suggests a complex relationship between host cell autophagy and fungal infections. Autophagy occurs in macrophages incubated with fungi, and the formation of LC3-positive phagosomes also occurs, which plays an important role in macrophage signaling and function in response to fungi [28]. In particular, gut commensal yeast-recognized dectin-2 induces LC3-associated phagocytosis, and influences secretion of the inflammatory cytokines TNF-α and IL-1β in BMDCs [29]. However, autophagy in BMDCs after particulate β-glucans (WGP) derived from the yeast *Sacchromyces cerevisiae* treatment had not been examined, and whether autophagy could be induced in DCs upon WGP treatment had not been determined. In this report, we found that WGP could induce the LC3 recruitment by increasing LC3-II expression. Moreover, we also found autophagosome formation in WGP-induced BMDCs, which indicated that, upon WGP binding to dectin-1 in DCs, autophagy is triggered in addition to the LC3-associated phagocytosis.

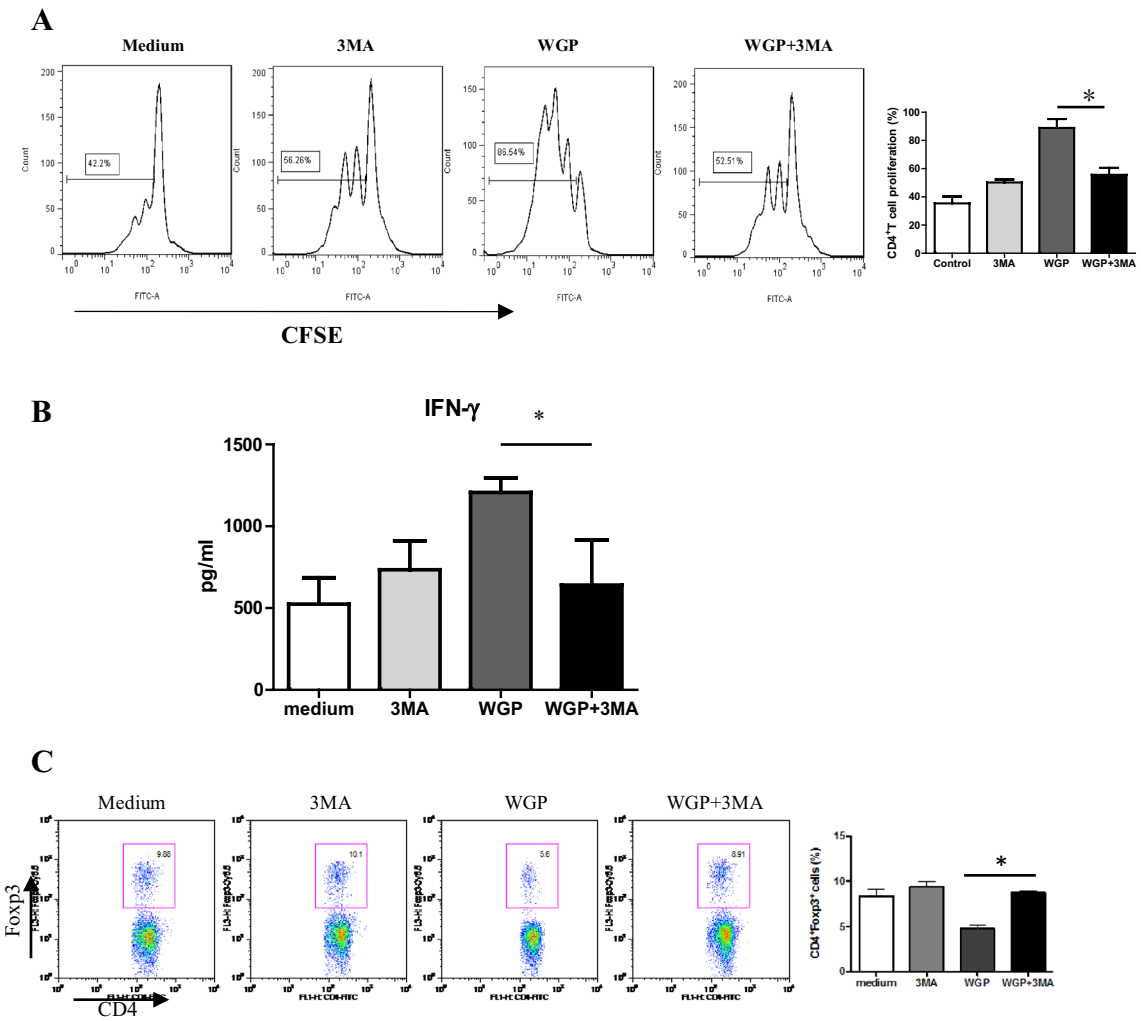

Fig. 4 Autophagy deficiency altered CD4 + T-cell proliferation and differentiation stimulated by WGP-activated BMDCs. a Immature BMDCs were incubated with WGP for 48 h, either with or without 3MA, washed to clear residual 3-MA, and subsequently co-cultured with CFSE-labeled OVA-Tg CD4 + T cells at a 1:10 ratio in the presence of OVA for 3 days. b Supernatants from cell culture medium were collected and the IFN-γ production was detected by ELISA. c CD4 + Foxp3 + T cells are shown by intracellular staining. Results are from one representative of three performed experiments and the data from three independent experiments are shown as mean ± SD. The asterisk (*) indicates $p < 0.05$ for comparisons between WGP with or without 3MA for both graphs

One of the major pathways in which a novel role for autophagy has been described is antigen presentation by major histocompatibility complex class II molecules (MHC-II). In the present study, autophagy inhibition significantly decreased the expression of MHC-II and CD86 in WGP-induced BMDCs. CD86 is an important co-stimulatory molecule of DCs for T-cell stimulation. DCs presenting antigen and activating T cells require three signals: (1) the recognition of MHC complexes on DCs by Ag-specific T-cell receptors in the DC–T-cell interaction, (2) DC–T-cell clustering, which is the interaction between co-stimulatory molecules expressed by DCs and their ligands expressed by T cells [30], and (3) cytokines, which modulate T-cell activation [31]. Therefore, we showed that autophagy increased the expression of co-stimulatory molecules to enhance the antigen presentation of WGP-induced DCs. In addition, autophagy promoted the production of TNF-α by WGP-induced DCs, but did not influence IL-12 secretion, which suggests that maturation of autophagy-induced DCs may have a unique pathway. Moreover, downregulated expression of iNOS in autophagy-deficient DCs induced by WGP supported this hypothesis. Although autophagy affects the antigen presentation by MHC-II in DCs, we determined that autophagy deficiency attenuated the proliferation and differentiation of CD4 + T cells stimulated by WGP-induced DCs. After WGP treatment, BMDCs induced CD4 + T-cell proliferation to a greater extent than autophagy-deficient BMDCs. Autophagy-deficient BMDCs showed significantly less

IFN-γ production by co-cultured CD4 + T cells in response to WGP and promoted the differentiation of CD4 + T cells into Treg cells.

In summary, we report a novel mechanism by which autophagy is involved in regulating the co-stimulatory molecule expression, Th1 cytokine production, and CD4 + T-cell priming of WGP-induced DCs. A key area of further investigation will be to establish whether autophagy possesses clinical value for cancer immunotherapy.

Acknowledgements This work was supported by National Natural Science Foundation of China (81672799 to C.Q. and 31500731 to Y.N.) and the Changzhou Sci & Tech Program (CJ20160017 to J.D.).

Compliance with ethical standards

Conflict of interest The author's declare that they have no conflict of interest.

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